trichrome staining 4 weeks after MI. Biochemical assays were conducted in the frozen ventricular tissues or in the cultured cardiomyocytes.

RESULTS SphK2 inhibition significantly increased mortality after MI (Vehicle 20.3% vs. ABC 50.1%). ABC administration obviously exacerbated cardiac dysfunction, interstitial fibrosis, and myocardial apoptosis following MI. SphK2 inhibition upregulated the expression of remodeling marker genes (ANP, BNP, and β-MHC) in the myocardium. Higher plasma BNP levels in ABC-treated mice indicated more severe heart failure progression. Compared with vehicle-treated mice, myocardial HDAC activities were elevated in ABC-treated group. In the cultured cardiomyocytes, β1 adrenergic receptor agonist-induced apoptosis and remodeling genes expression were aggravated by SphK2 knockdown or ABC treatment in the cardiomyocytes, which was blocked by HDAC inhibitors.

CONCLUSIONS SphK2 and its product S1P exert protective roles in the remodeling processes and heart failure progression after MI potentially by inhibiting myocardial HDAC activation.

GW26-e0103

CD51 Positive Cardiac Cell Repair the Heart After Myocardial Infarction Through Transdifferentiation

Wanwen Lin, Chaoquan Peng, Ke Yang, Yang Chen
Department of Cardiology, the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

OBJECTIVES Acute myocardial infarction (AMI) is one of the most serious diseases that threaten human's health. AMI may lead to myocardiolysis, reconstruction and even heart failure. Many experiments demonstrated the potentiality of cardiac stem cells (CSCs) to treat AMI. They found evidence for enhanced revascularization of the infarct zone in stem cells-transplanted hearts, and even differentiate into functional myocytes. A variety of surface markers have been used to define CSCs. Until now, we do not virtually know the unified and specific surface markers of CSCs. Recently, the intergrin αV ($CD51^+$) are found to express on testicle and myocardial cells. They can proliferate and differentiate into cardiomyocytes and endothelial cells. The aim of this study was to investigate the effects of CD51 positive cardiac cells on left ventricular dysfunction or heart failure after AMI.

METHODS CD51 positive cardiac cells were isolated from 7days old C57BL/6 mice. The cells were labeled by transfected with red fluorescent protein(RFP) before transplant. Wild- type C57BL/6 mice underwent myocardial infarction by ligating the left anterior descending coronary artery with injection of saline (n=7), or RFP-CD51+ cardiac cells (n=7). Cardiac function was monitored using echocardiography after 1 week and 4 weeks. The hearts were harvested and frozen at 4w after cell implantation. Myocardial tissue sections were traced and stained with immunofluorescence to find cells which glowing red fluorescent and alpha sarcomeric actin positive under fluorescence microscope.

RESULTS Transplantation of CD51 positive cardiac cells into AMI mouse hearts improved cardiac function and remodeling, as determined by echocardiography. Compare with the control group, there were higher left ventricular ejection fraction (35.93±12.09% VS 16.59±1.88%, $p<0.001$), shorten fraction (17.30±6.35% VS 7.51±0.81%, $p<0.001$) and smaller left ventricular end-diastolic volume (59.73±30.84ul VS 118.16±41.37ul, $p<0.05$) in the experimental group by 1w. The cell treatment group remained significantly improved relative to saline control animals at 4w. The immunofluorescence detection of cell treatment group showed RFP-CD51+ cells are alpha sarcomeric actin positive which indicate the transplantation cells can survive in myocardial infarction regions and differentiate into mature cardiocyte.

CONCLUSIONS The intramyocardial delivery of CD51 positive cardiac cells after AMI can improve cardiac function, and attenuate remodeling at all time points. The in vivo differentiated CD51 positive cells expressed cardiac markers, as determined by immunohistochemisty.

GW26-e0202

Expression of Matrix Metalloproteinase-2 and Mitogen-Activated Protein Kinases in Ascending Aortic Aneurysms and Aortic Valves of Patients with Bicuspid or Tricuspid Aortic Valves: a Comparative Study

Chunmao Wang, Xiaogang Sun, Xiangyang Qian, Qian Chang
State Key Laboratory of Cardiovascular Disease, Aorta Surgery Center, Fuwai Hospital, National Center for Cardiovascular Diseases

OBJECTIVES The objective of this study was to compare the expression of matrix metalloproteinase-2 (MMP-2) and mitogen-activated protein kinases (MAPKs) pathway molecules in ascending thoracic aortic aneurysms (ATAAs) of patients with bicuspid and tricuspid aortic valves.

METHODS Ascending aortic aneurysmal wall specimens and valve samples were taken from tricuspid aortic valves (TAV) patients (n=20, 51.5±7.6 years) and tricuspid aortic valves(BAV)patients (n=20, 47.4±6.3 years) when surgery repair. Normal ascending aortic specimens (n=20) were obtained from 20 patients with coronary heart diseases (CHD) undergoing CABG without aortopathy. MMP-2 expression was examined by qPCR, Western blot in aortic and valve tissues. MAPKs were measured by Western blot in aortic and valvular specimens. Some aneurysmal walls were stained with HE and EVG.

RESULTS By histological examination all aortic aneurysm walls showed TAV aneurysms patients exhibited severe elastin fragmentation and markedly decreased elastin and much collagen deposition, compared with BAV aneurysms. Aortas from the BAV group displayed intact and regular arrayed elastic fibers in the intima and media and very few collagens. MMP-2 expression was greater in valves and aortic aneurysm tissues from ATAAs associated with bicuspid valves when compared with those from tricuspid valves, irrespective of the level of mRNA and protein ($p<0.05$). In the aneurysmal wall specimens, t-p38 MAPK was higher in TAV patients than that in BAV patients and CHD patients ($p<0.05$). The p-p38 level was also increased in TAV aneurysmal aortic walls compared with BAV group. However, there is no significant difference in t-p38 MAPK between BAV patients and CHD patients ($p>0.05$). In addition, t-p38 and p-p38 MAPK levels had also no significant differences between BAV and CHD patients. Additionally, No statistical differences were found in p-ERK1/2, t-ERK1/2 and p-ERK/t-ERK1/2 between these three groups. Compared with normal aortic group, p-JNK, t-JNK and p-JNK/t-JNK were increased in two aneurysmal groups associated with BAV and TAV ($p<0.05$). Additionally, the BAV patients have increased levels of t-p54JNK and p-p54JNK compared with TAV patients. In the valvular tissues, p-p38, t-p38 and p-p38/t-p38 MAPK were no significant differences between BAV and TAV patients. Additionally, t-ERK1/2 level was higher in TAV patient than in BAV patient while p-ERK1/2 and p-ERK/t-ERK1/2 levels had no statistical differences between TAV and BAV patients. Compared with BAV patients, the levels of p-JNK, t-JNK and p-JNK/t-JNK were increased in TAV patients ($p<0.05$).

CONCLUSIONS The up-regulation of MMP-2 in aneurysms associated with BAV may partly elucidate the predilection to aneurysm formation in these patients compared with TAV. Furthermore, in the BAV associated with ascending aortic aneurysms, increased p54JNK level was found, which may contribute to elucidate the elevated level of MMP-2 compared with TAV associated with ascending aortic aneurysms.

GW26-e0203

Effects of Nerve Growth Factor on Late Reperfusion after Myocardial Infarction

Yang Liu, Jinhui Wu
Department of Geriatrics, West China Hospital of Sichuan University, Chengdu, China

OBJECTIVES Nerve growth factor (NGF) is one of the most important bioactive molecules in nervous system, which involved in neuronal differentiation, development, repair and adjusting their general functions. Current studies have demonstrated that NGF plays a protective role in myocardial infarction and early reperfusion by reducing the myocardial cell apoptosis and improving ventricular remodeling. In the study, we investigated the role of nerve growth factor on late reperfusion by detecting cardiac structure and function with echocardiography.

METHODS Rats were randomly divided into four groups: Adenoviral vector group (Adv group, intramyocardial injection, adenoviral vector 10μl in total), NGF overexpression group (NGF group, constructing the adenovirus vector Ad-NGF containing nerve growth factor gene, 10ul Ad-NGF as previously described). Sham group and LR group (10μl normal saline as previously described, respectively). The models of late reperfusion (LR group, Adv group, NGF group) were established by ligating the anterior descending branch of coronary artery in anaesthetized rats, then loosening the ligature after 2 hours, and starting to reperfusion, the Sham group underwent thoracotomy without coronary ligation. On the 3^{th}, 7^{th}, 14^{th} and 28^{th} day after operation, 5 rats in each group were sacrificed and their hearts were harvested. The expression of NGF protein was examined with immunohistochemisty technology, cardiac structure and function were measured with echocardiography.

RESULTS On the 14th and 28th day, increased LVEDD, LVEDV, LVESV and decreased LVEF, FS were encountered in the LR group. On the 14th, 28th day, the NGF group had higher LVEF,FS levels compared to LR group. On the 28th day, the NGF group had lower LVEDD, LVESD, LVEDV, LVESV levels compared to LR group.

CONCLUSIONS The effectiveness of exogenous NGF may help postpone the myocardial remodeling and promote the heart function and myocardial blood flow by ameliorated (endothelial cells) ECs and cardiomyocyte survival and promoting the myocardial neovascularization.

GW26-e0748

The Present and the Probable Future Rat Models of Myocardial Infarction

Tian Zuo,[1] Wenhui Li,[1] Lu Jiang,[1] Liheng Guo[2]
[1]The Second Clinical Medical School, Guangzhou University of Chinese Medicine, Guangzhou, China; [2]Intensive Care Unit, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou, China

OBJECTIVES To review the current research and published literature regarding the development of rat models of Myocardial Infarction (MI) and give a comprehensive evaluation on them.

METHODS A review of the current and relevant papers and research information via a search of several large databases of medical from January 1980 to present.

RESULTS Comparing with other animals like pig or monkey, rats have advantages on feeding and related cost. This model of MI has been the dominant choose of the experimental study by taking up 70% or even higher proportion in this field. With the progressing of science and technology, some new methods in rat MI models such as balloon occlusion and gene knockout are spring up in these years. However, the seemingly old method - coronary artery ligation is still be the first choice of most researchers. Isopropyl adrenaline induced model is also usually been adopted too. More recent efforts have been aimed at making those mature and classic methods more perfect.

CONCLUSIONS Rat model of MI can be relatively quickly made now by ligation of left anterior descending branch of the coronary artery, and its mortality has decreased effectively based on existing researches. This model will continue to be a priority for researchers in a long period of time.

GW26-e0777

Effects of Rosuvastatin on Tar and Nicotine Induced the Interaction Between Thrombomodulin and Thrombin by Live-Cell Single-Molecule Force Spectroscopy

Yujie Wei,[1] Bin Lai,[1] Xiaoli Shi,[2] HuiLiang Liu[1]
[1]Department of Cardiology, the General Hospital of Chinese People's Armed Police Forces; [2]National Center for Nanoscience and Technology

OBJECTIVES Tar and nicotine exposure via cigarette smoking and tobacco chewing is associated with vascular complications. Rosuvastatin has been marked in its effect on endothelial cells protection and anticoagulation. As a vital anticoagulation cofactor, thrombomodulin (TM) located on the endothelial cell surface is able to regulate intravascular coagulation by binding to thrombin, and the binding results in thrombosis inhibition. The present study investigated the effects of Rosuvastatin on tar and nicotine induced the interaction between TM and thrombin and the expression of TM.

METHODS We have applied the advanced method of live-cell single-molecule force spectroscopy to investigate the effects of Rosuvastatin on tar and nicotine induced the interaction between TM and thrombin. RT-PCR and Flow cytometry techniques were used for detecting TM mRNA and protein. Microplate Reader was used for detecting TM activity.

RESULTS Our results showed that the single-molecule binding force of thrombomodulin and thrombin detected by AFM in the living cells was about 55 pN and the binding probability was about 27.5%. Tar significantly decreased the binding probability between TM and thrombin (3.75 +/- 3.02)% when compared with that of control group (26.46 +/- 5.35) % ($P < 0.05$), while Rosuvastatin significantly reversed the binding probability(29.7 +/- 5.07) % induced by tar. And Rosuvastatin significantly enhanced the binding force between TM and thrombin (80.8 +/- 15.39) PN when compared with that of control group (56.8 +/- 8.39)PN ($P < 0.05$).

Flow cytometry and RT-PCR results revealed that rosuvastatin and tar increased the expression of TM in HUVECs and both have an additive effect. Microplate Reader results revealed that tar significantly decreased the TM activity while rosuvastatin could inhibited this effect. And rosuvastatin itself also increased the TM activity. However nicotine was no effect on the above indices of TM.

CONCLUSIONS Tar may inhibit the activity of TM though decreasing the binding probability between TM and thrombin. Rosuvastatin may prevent tar-induced the decreasing of TM activity by increasing TM expression, reversing the binding probability between TM and thrombin and enhancing the binding force between TM and thrombin. However nicotine was no effect on the activity of TM. This study provided a new approach and new evidence for studying the mechanism of thrombosis triggered by cigarette smoking and the anticoagulation mechanism for rosuvastatin.

GW26-e0784

CD137/NFATc1 Signaling Modulates Neointima Formation and the Phenotype Transformation of Vascular Smooth Muscle Cells Through miR-145

Wei Zhong, Jinchuan Yan
Department of Cardiology, Affiliated Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, P. R. China 212001

OBJECTIVES Vascular smooth muscle cell (VSMC) phenotype transformation is an important phenomenon of vessel neointima in lesion progression. CD137 can accelerate the plaque formation, but the detailed mechanisms are unclear. Thus, we investigated the effect of CD137 signaling on VSMC phenotype transformation as well as the potential underlying mechanism.

METHODS Agonist-CD137 antibody and anti-CD137 antibody were used to activate or block CD137 axis respectively in $ApoE^{-/-}$ mice or VSMC. The content of miR-145, NFATc1 and phenotype marker was measured by RT-PCR and western blot. Immunofluorescence was used to observe the distribution of NFATc1 or nuclear translocation. VSMCs were transfected with miR-145a-5p mimics or inhibitors by Lipofectamine. Eukaryotic expression vector and luciferase vector were constructed and co-transfected to the 293T with mimics or inhibitors to measure the protein level and fluorescence intensity respectively. Stable VSMC cell line which knock-down NFATc1 or overexpress miR-145 and control were built by lenti-virus. CCK-8 assay and transwell assay were performed to detect the proliferation and migration of cell. miR-145 agomir and agonist CD137 antibody were used in $ApoE^{-/-}$ mice and Immunohistochemistry or Masson's trichrome assay were performed to observe the plaque stability.

RESULTS miR-145a-5p expression was downregulate (0.28±0.06 VS 1.00±0.00, 0.28±0.06 VS 0.67±0.13, $p<0.05$), whereas expression of nuclear factor of activated T-cells c1 (NFATc1) was significantly upregulated (2.21±0.21 VS 1.47±0.13, $p<0.05$). Phenotype markers such as SM-MHC, α-SMA and vimentin were also altered in vivo or in vitro upon treatment with agonistic CD137 antibody (0.28±0.03 VS 0.540±0.12, 0.18±0.04 VS 0.66±0.08, 0.34±0.05 VS 2.42±0.23, $p<0.05$). Overexpression of NFATc1 by plasmid transfection demonstrated that NFATc1 independently contributed to phenotype modulation in VSMC. Otherwise, dual-luciferase report assay and mimic/inhibitor transfection demonstrated that NFATc1 was the target gene of miR-145 (0.41±0.05 1.00±0.00, 0.36±0.07 VS 1.00±0.00, $p<0.05$). Inhibition of miR-145a-5p upregulated overexpression of miR-145a-5p but downregulated NFATc1 in vitro. Both overexpression of miR-145 and knockdown NFATc1 suppressed the proliferation and migration of VSMC and inhibited the phenotype transformation of VSMC induced by CD137. Up-regulating miR-145 through agomir in $Apoe^{-/-}$ mice significantly reduced atherosclerotic lesion areas induced by CD137 in multi ways.

CONCLUSIONS Results indicate that CD137/NFATc1 pathway plays an important role in regulating VSMC phenotype transformation and miR-145 directly regulates NFATc1 expression in CD137 signaling.

GW26-e0799

Effect of CD137 Signaling on the Expression of Nuclear Factor of Activated T-Cells, Cytoplasmic 1(NFATc1) in Mice VSMCs Through TRAF6/NF-κB Pathway

Yunjie Yin, Jinchuan Yan
Department of Cardiology, Affiliated Hospital of Jiangsu University

OBJECTIVES To observe whether the CD137 signaling affect the expression of NFATc1 in mice VSMCs through TRAF6/NF-κB pathway.

METHODS Patch-attaching method was used for primary culture of mouse aortic vascular smooth muscle cells(VSMCs). Immunofluorescence was uesd to identify the primary cells. The expression of CD137 mRNA and NFATc1 mRNA were measured by real-time quantitative